

# Role of Aerobic and Anaerobic Circular Mantle Muscle Fibers in Swimming Squid: Electromyography

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**Abstract.** Circular mantle muscle of squids and cuttlefishes consists of distinct zones of aerobic and anaerobic muscle fibers that are thought to have functional roles analogous to red and white muscle in fishes. To test predictions of the functional role of the circular muscle zones during swimming, electromyograms (EMGs) in conjunction with video footage were recorded from brief squid *Lolliguncula brevis* (5.0–6.8 cm dorsal mantle length, 10.9–18.3 g) swimming in a flume at speeds of 3–27 cm s<sup>-1</sup>. In one set of experiments, in which EMGs were recorded from electrodes intersecting both the central anaerobic and peripheral aerobic circular mantle muscles, electrical activity was detected during each mantle contraction at all swimming speeds, and the amplitude and frequency of responses increased with speed. In another set of experiments, in which EMGs were recorded from electrodes placed in the central anaerobic circular muscle fibers alone, electrical activity was not detected during mantle contraction until speeds of about 15 cm s<sup>-1</sup>, when EMG activity was sporadic. At speeds greater than 15 cm s<sup>-1</sup>, the frequency of central circular muscle activity subsequently increased with swimming speed until maximum speeds of 21–27 cm s<sup>-1</sup>, when muscular activity coincided with the majority of mantle contractions. These results indicate that peripheral aerobic circular muscle is used for low, intermediate, and probably high speeds, whereas central anaerobic circular muscle is recruited at intermediate speeds and used progressively more with speed for powerful, unsteady jetting. This is significant because it suggests that there is specialization and efficient use of locomotive muscle in squids.

## Introduction

Fishes have three myotomal muscle types, that is, red, pink, and white fibers, that are structurally, metabolically, and functionally distinct. The red muscle is characterized by a high myoglobin content, extensive capillary beds, a high density of mitochondria, and high levels of oxidative enzymes (Bone, 1966; 1978). It operates aerobically, fatigues slowly, has low rates of activation and relaxation, and has low shortening velocities (Johnston *et al.*, 1977). White muscle is characterized by a high myofibrillar density, a low content of mitochondria, poor blood supply, and high levels of glycolytic enzymes (Flitney and Johnston, 1979). It operates anaerobically, fatigues rapidly, has high rates of activation and relaxation, and has high shortening velocities (Curtin and Woledge, 1988). Pink muscle is intermediate in structure, metabolism, and contractile properties between red and white muscle (Johnston *et al.*, 1977; Coughlin *et al.*, 1996). Generally, red muscle is recruited for the lowest, steady, undulatory swimming speeds (Rome *et al.*, 1984); pink muscle is recruited at intermediate swimming speeds (Johnston *et al.*, 1977); both red and pink muscle are recruited at maximum steady swimming speeds (Coughlin *et al.*, 1996); and white muscle is recruited at higher swimming speeds when swimming becomes unsteady (Rome *et al.*, 1984; Jayne and Lauder, 1996).

Squids and cuttlefishes have muscle types in the mantle musculature similar to red and white muscle. The mantle musculature of cephalopods functions as a constant volume system with a three-dimensional array of tightly bundled muscles commonly called a muscular-hydrostat (Kier and Smith, 1985; Smith and Kier, 1989). During jet propulsion, circular muscles within the mantle contract, which consequently decreases mantle diameter and forces intramantle water out of the funnel. Because changes in mantle length during contraction are negligible and the mantle muscula-

ture is constant in volume, contraction of circular muscle results in concomitant thickening of the mantle wall. Obliquely oriented collagen fibers traversing the thickness of the mantle wall are strained and store energy. This energy together with contraction of radially oriented muscle fibers, which extend from the inner to outer surface of the mantle, power mantle refilling (Gosline and Shadwick, 1983; Gosline et al., 1983; Kier, 1988). The circular muscle responsible for mantle contraction within this system consists of three anatomically and metabolically distinct muscle layers when viewed in cross section: an inner, outer, and middle layer (Bone et al., 1981; Mommsen et al., 1981). The inner and outer (peripheral) layers are thin and rich in mitochondria; they have high succinic dehydrogenase (SDH) activity, a high ratio of oxidative to glycolytic enzyme activity, and heavy vascularization. The middle (central) layer is thick and poor in mitochondria; it has low SDH activity, a high ratio of glycolytic to oxidative enzyme activity, and sparse vascularization. Because of these structural and biochemical differences, Bone et al. (1981) and Mommsen et al. (1981) suggested that the inner and outer layers are analogous to aerobic red muscle in fish and are used for slow, steady swimming and rhythmic, respiratory contractions, while the central layer is analogous to anaerobic white muscle and is used during burst swimming, escape, and capture events. This is consistent with the classic view (Wilson, 1960) that squid switch between quiet "respiratory" jetting and giant fiber escape responses. However, O'Dor (1982, 1988a) and Bartol (1999) have reported fully graded swimming in squid swim-tunnel studies, and Otis and Gilly (1990) and Preuss and Gilly (2000) have shown that interplay between giant and non-giant axon systems, which innervate the circular mantle musculature of squid, allow for considerable flexibility in jetting behavior and may facilitate smooth speed transitions.

Although peripheral and central circular muscle have several structural and biochemical similarities to red and white muscle in fishes, the functional roles of circular muscle types during swimming in squids and cuttlefishes remain unresolved. Bone et al. (1994) found large-amplitude electromyographic activity in the mantle of cuttlefish *Sepia officinalis* during escape jetting and concluded that both the central anaerobic and peripheral aerobic circular muscles were active. However, since electrodes were not inserted exclusively in either muscle layer, the functional role of the circular muscle types is still uncertain. Accumulations of metabolic end products within mantle musculature of squids during routine behavior, exercise, and after exercise have been the focus of many studies (Hochachka et al., 1975; Grieshaber and Gade, 1976; Storey and Storey, 1978; Fields and Quinn, 1981; Pörtner et al., 1991, 1993; Finke et al., 1996), but no distinctions were made in those studies between end products found within the various layers of circular muscle. On the basis of mantle pressure measure-

ments, O'Dor (1988b) concluded that both types of circular muscle fiber must be active in *Illex illecebrosus* at subcritical swimming speeds to generate the necessary swimming power. Furthermore, Finke et al. (1996) suggested that *Lolliguncula brevis*, which tends to oscillate between high and low muscular activity at increased swimming velocities, relies on both types of circular muscle fiber at high speeds. However, once again, direct documentation of the functional roles of the two circular mantle muscle fibers is lacking.

In this study electromyograms (EMGs) were collected in conjunction with video footage to determine the role of the peripheral aerobic and central anaerobic circular muscle layers in brief squid *Lolliguncula brevis* swimming over a range of speeds. Two experiments are reported: in one, EMG activity was recorded from electrodes traversing both peripheral and central circular muscle layers; in the other, EMG activity was recorded from electrodes embedded exclusively in the central circular muscle layer. It was not possible to embed electrodes solely in peripheral circular muscle because the muscle layer was too thin (~0.1 mm) for reliable placement. However, by comparing results from the two experiments, it was possible to postulate the functional roles of the circular muscle zones.

## Materials and Methods

### *Experimental animals*

In August–October 1998, brief squid *Lolliguncula brevis* (Blainville) were captured by trawl within embayments along the seaside of Virginia's Eastern Shore and within the York River, Virginia. Squid captured along the Eastern Shore of Virginia were transported to the Virginia Institute of Marine Science (VIMS) Eastern Shore Laboratory in Wachapreague, Virginia; squid captured in the York River were transported to the VIMS main campus in Gloucester Point, Virginia. Squid were kept alive in the field using 120-quart coolers equipped with filtration and aeration systems, which were powered by 12-V, sealed, gel-cell batteries. At Wachapreague and Gloucester Point, squid were kept in flow-through raceway tanks for at least one week prior to experimentation and fed a diet of grass shrimp *Palaemonetes pugio*. Experiments on 12 squid (3.5–7.8 cm dorsal mantle length [DML], 4.3–27.6 g) were performed, but results from only 5 squid (5.0–6.8 cm DML, 10.9–18.3 g) are reported here. Some squid were eliminated from consideration because of one or more of the following: (1) the location of the electrodes in the muscle was somewhat uncertain, (2) ambient electrical noise was excessive, (3) the squid was not cooperative in swim tunnels.

### *Electromyogram (EMG) recordings*

Disposable, paired hook-wire electrodes (150- $\mu$ m-gauge insulated nickel alloy, Nicolet Biomedical, Madison, WI)

were used for EMG recordings. The hook portions of the manufactured electrode wires were too large for recording EMGs from the peripheral and central circular muscle layers, which were about 0.1 mm and 1.0 mm thick, respectively. Therefore, the hook portions of the electrodes were modified so that they were 1.0 mm in total length with 0.5 mm of insulation removed at the tip. The squid were anesthetized ( $\sim 2$  min) in an isotonic solution of  $\text{MgCl}_2$  (7.5%  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ) and seawater (Messenger *et al.*, 1985), and each pair of electrodes was inserted obliquely, using a hypodermic needle, into the lateral mantle wall at a point 60% of the mantle length from posterior. Electrode spacing within the muscle was about 2–6 mm. Insertion of two pairs of electrodes for simultaneous recording from different muscle groups was attempted on several occasions, but these attempts were unsuccessful because dissections revealed that at least one pair of electrodes was not in target muscle fibers or the two sets of electrodes interfered with swimming. Thus, recordings from only one pair of electrodes during each experiment are reported.

The electrode pair from which EMGs were recorded was embedded in either the central circular muscle layer or both the central circular muscle layer and the peripheral circular muscle layer adjacent to the skin. It was not possible to embed electrodes exclusively in the peripheral layer of circular muscle because it was too thin ( $\sim 0.1$  mm) for hook-wire electrodes. Fine wire needle electrodes like those described in Kier *et al.* (1989), which allow for very precise electrode placement, were not used because of difficulties associated with anchoring the electrodes to the mantle of *L. brevis* and the high probability that anchoring tools (*e.g.*, clamping blocks) would disrupt swimming. During electrode placement, no attempt was made to avoid radial muscles, which extend from the inner to outer mantle surface and partition the circular muscle into 0.1-mm sections. Electrical activity of radial muscles, however, did not obscure electrical activity from the circular muscle layers, because radial muscles are active during mantle expansion, whereas circular muscles are active during mantle contraction (Gosline and Shadwick, 1983; Gosline *et al.*, 1983). To help ensure reliable placement of electrodes, many practice insertions were performed on preserved squid prior to the experiments, and depth references were marked on hypodermic needles used for implantation. Sufficient slack in the electrode wires was provided so that anchoring electrodes at other locations along the body was not necessary to prevent dislodgment.

After successful implantation, squid were placed in a  $20 \times 10 \times 10$  cm holding section within a 16-l recirculating water tunnel (Vogel and LaBarbera, 1978) filled with aerated seawater (24‰, 22 °C) and allowed to recover from surgery at low flow velocity ( $1 \text{ cm s}^{-1}$ ). Flow velocity in the tunnel was controlled using two propellers arranged in a rotor-stator configuration and a  $\frac{1}{4}$  hp variable-speed motor, which was shielded using aluminum sheeting to reduce radiated electromagnetic noise. A ground electrode was attached to a down-

stream collimator within the flume. The ground electrode together with the embedded bipolar hook-wire electrodes, which were attached to insulated Nicolet micrograbbers, were plugged into a two-channel differential amplifier using Nicolet DIN 42 802 connectors. The differential amplifier was part of a Nicolet Compass II PortaBook system, which is designed to record electromyograms and somatosensory, auditory, and visual evoked potentials from human patients. The PortaBook was used to record, amplify, and filter electromyograms collected at various frequencies. For this study, EMGs were collected in the free-run mode using a bandpass filter of 20–10,000 Hz, which was the most effective Nicolet filter available for electromyographic recordings, and all EMG traces were recorded on the hard drive.

After the squid became active in the holding section, flow velocity within the flume was increased to  $6 \text{ cm s}^{-1}$  and the animal was allowed to acclimate to flow, which generally occurred within 15 min. After the acclimation period, squid were exposed to a flow velocity of  $3 \text{ cm s}^{-1}$  for 10 min. Speed was subsequently increased by  $3 \text{ cm s}^{-1}$  every 10 min until the squid could no longer keep pace with free-stream flow. During each of the 10-min speed increments, EMGs were collected. Because of limitations of the PortaBook, it was not possible to record EMG signals on a channel of a video recorder or precisely timelock video and EMG recordings. Therefore, to provide a synchronized record of EMG responses and swimming behavior, the computer screen of the PortaBook was positioned below the holding section of the flume, and both Nicolet display output and swimming behavior of the squid were videotaped simultaneously from a lateral perspective using a Sony Hi-8 video camera. After squid were exhausted and could no longer maintain free-stream velocity, they were removed from the flume and over-anesthetized in an isotonic solution of  $\text{MgCl}_2$  (7.5%  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ) and seawater. The squid then were transferred to 10% buffered formalin for later dissection to determine precise electrode location. Some cooperative squid were not euthanized after the initial recording session. Instead, the electrode wires were cut, leaving the embedded section undisturbed within the mantle so that it could be examined later to determine placement, and the squid were returned to the raceway tanks. After several hours these squid were retrieved, electrodes were embedded in a different circular muscle layer, and EMGs were recorded during another swimming session. Following the completion of the second swimming session, these animals were over-anesthetized, fixed, and dissected as described above to determine electrode placement.

#### *Kinematic analysis*

Video footage of trials in which squid were cooperative at all swimming speeds, ambient electrical noise was low, and electrode placement was unambiguous was analyzed using a

Sony EVO-9700 editing deck and a Peak Motus video and computer motion measurement system (Peak Performance Technologies, Englewood, CO). At each speed several video sequences with clear mantle profiles and representative EMG recordings were selected. Mantle diameter at the location of electrode placement, swimming velocity, and acceleration of the squid were measured for each frame of video ( $30 \text{ frames s}^{-1}$ ) within the sequences, and frames where EMG responses began and ended were marked using the event feature in the Peak Motus system. Mantle diameter, velocity, and acceleration data were smoothed using a fourth-order Butterworth filter to account for video jitter and digitization error. Peak video data and Nicolet EMG data were imported into Microsoft Excel and aligned using event markers from the Peak file and timebase frequencies from both files. Synchronization of the data was reliable only to within 33.3 ms because the video camera records at 30 frames  $\text{s}^{-1}$ . Since the objective of this study was simply to determine the presence or absence of EMG activity within the circular muscle layers during mantle contractions, more precise analysis of the timing of EMG activity was unnecessary.

## Results

The five specimens of *Lolliguncula brevis* (5.0–6.8 cm DML) considered in this study matched free-stream flow well at speeds less than  $21 \text{ cm s}^{-1}$ , but at higher speeds they had some difficulty matching flow velocity and eventually collapsed against the downstream weir. Prior to collapse, the squid accelerated and decelerated erratically, and the contraction frequencies were more irregular than those observed at lower velocities. At low ( $3\text{--}9 \text{ cm s}^{-1}$ ) and intermediate ( $12\text{--}18 \text{ cm s}^{-1}$ ) swimming velocities, squid relied on both fin and jet propulsion, whereas at high subcritical speeds ( $21\text{--}27 \text{ cm s}^{-1}$ ), they relied exclusively on jet propulsion and, like *Loligo opalescens* and *Illex illecebrosus* (O'Dor, 1988a), wrapped their fins against the mantle. The electrodes did not appear to impede swimming in the five squid considered; however, some squid, which were excluded from this analysis, were visibly agitated after surgery, swam inconsistently at most swimming speeds, and frequently swam forcibly into the flume sides.

The amplitude and frequency of electrical activity varied from animal to animal, but the basic patterns of activity presented in this paper are representative of all the squid considered. Results from a squid with electrodes inserted into the central and peripheral circular muscle layers (Fig. 1) show significant EMG activity at low ( $6 \text{ cm s}^{-1}$ ), intermediate ( $15 \text{ cm s}^{-1}$ ), and high speeds ( $24 \text{ cm s}^{-1}$ ); this activity was correlated with mantle contraction. The amplitude of EMG activity increased with speed and, as was typical of other squid examined, frequency of mantle contraction was greater at the highest speed tested ( $24 \text{ cm s}^{-1}$ ) than at low

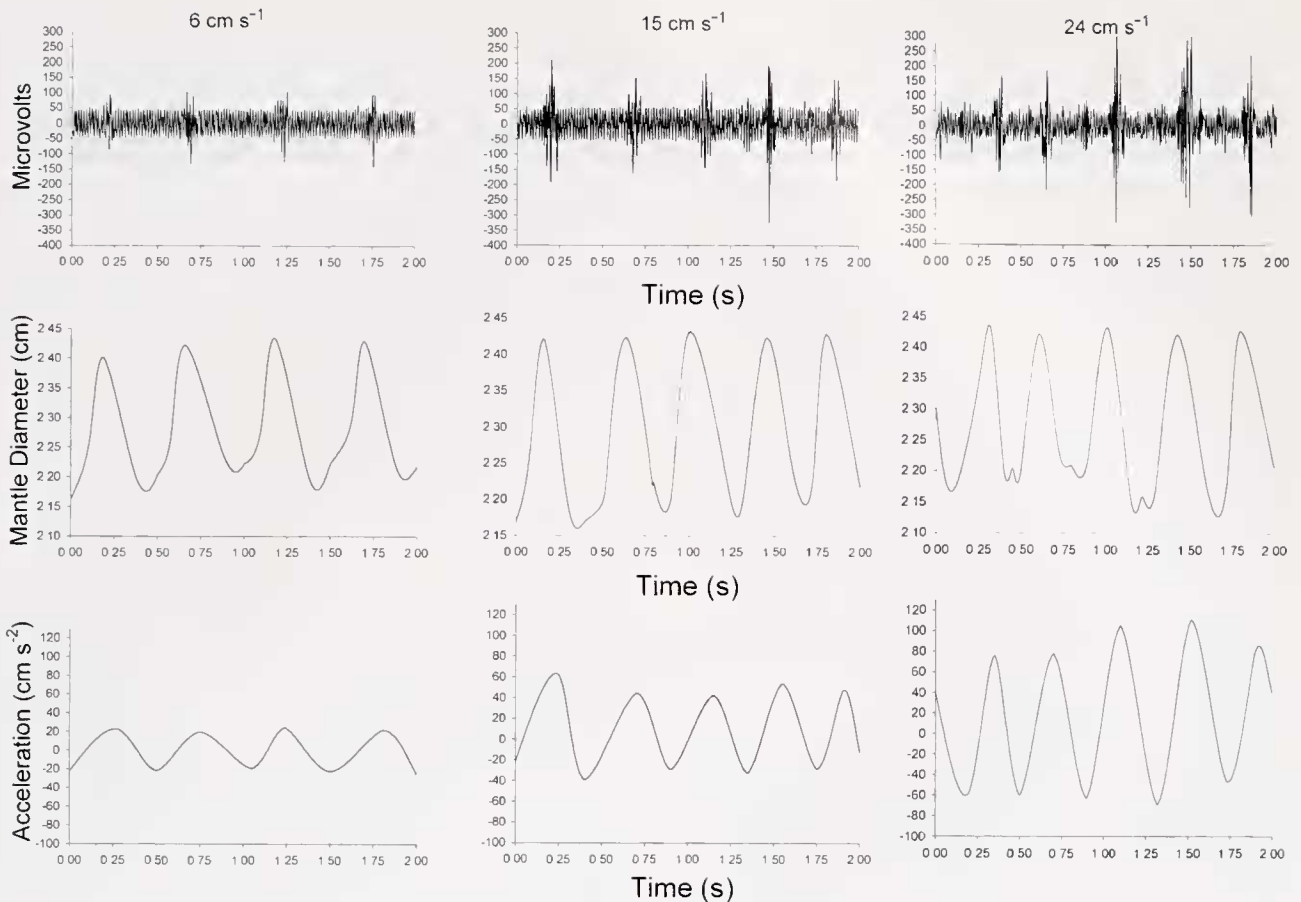
speed ( $6 \text{ cm s}^{-1}$ ). Moreover, maximum accelerations throughout the jet cycle were closely coupled with high-magnitude EMG bursts recorded during mantle contraction. At high speeds ( $24 \text{ cm s}^{-1}$ ), lower amplitude electrical activity sometimes occurred during mantle expansion.

Electromyographic recordings from a squid with electrodes embedded in only central circular muscle (Fig. 2), but with settings for bandpass filter, sensitivity, timebase, etc., identical to those used for recordings from both central and peripheral layers, show no EMG activity during mantle expansion and contraction at  $6 \text{ cm s}^{-1}$ . At an intermediate speed of  $15 \text{ cm s}^{-1}$ , occasional EMG activity was detected. (The mean central circular muscle recruitment speed for all squid considered was  $15.3 \pm 3.7$  [SD]  $\text{cm s}^{-1}$ .) Most of the large-amplitude electrical activity at intermediate speeds occurred during the contraction phase of the jet cycle, but sometimes smaller amplitude activity occurred during mantle expansion (Fig. 2). Furthermore, the large-amplitude EMGs at intermediate speeds were coupled with large changes in mantle diameter and high peaks of acceleration. Generally, the frequency of EMG bursts increased with speed up to maximum speeds of  $21\text{--}27 \text{ cm s}^{-1}$ , when muscular activity coincided with the majority of mantle contractions. Again large-amplitude signals correlated with mantle contraction, whereas less frequent low-amplitude signals correlated with mantle expansion. Contraction amplitude and frequency were more irregular at  $24 \text{ cm s}^{-1}$  than at lower velocities, and high-amplitude EMG peaks were strongly coupled with high accelerations.

## Discussion

The results of this study indicate that the peripheral aerobic and the central anaerobic circular muscle fibers in squid probably have distinct functional roles during swimming. Electrodes implanted in both peripheral and central circular muscle fibers recorded EMG activity with each mantle contraction at all swimming speeds ( $3\text{--}27 \text{ cm s}^{-1}$ ). However, electrodes implanted exclusively in central (anaerobic) muscle did not record EMG activity during mantle contraction until speeds of about  $15 \text{ cm s}^{-1}$ , when activity was sporadic. At speeds above  $15 \text{ cm s}^{-1}$ , the frequency of electrical activity increased with swimming speed up to maximum speeds of  $21\text{--}27 \text{ cm s}^{-1}$ , when anaerobic muscular activity coincided with the majority of mantle contractions and with high accelerations. These results indicate that peripheral aerobic circular muscle is used for slow, intermediate, and probably high speeds, whereas central anaerobic muscle is recruited at intermediate speeds and used progressively more with increasing speed for powerful, unsteady jetting typical of higher speed swimming.

The observed periodic detection of central anaerobic circular muscle activity at intermediate speeds is interesting given that anaerobic muscle is often associated only with

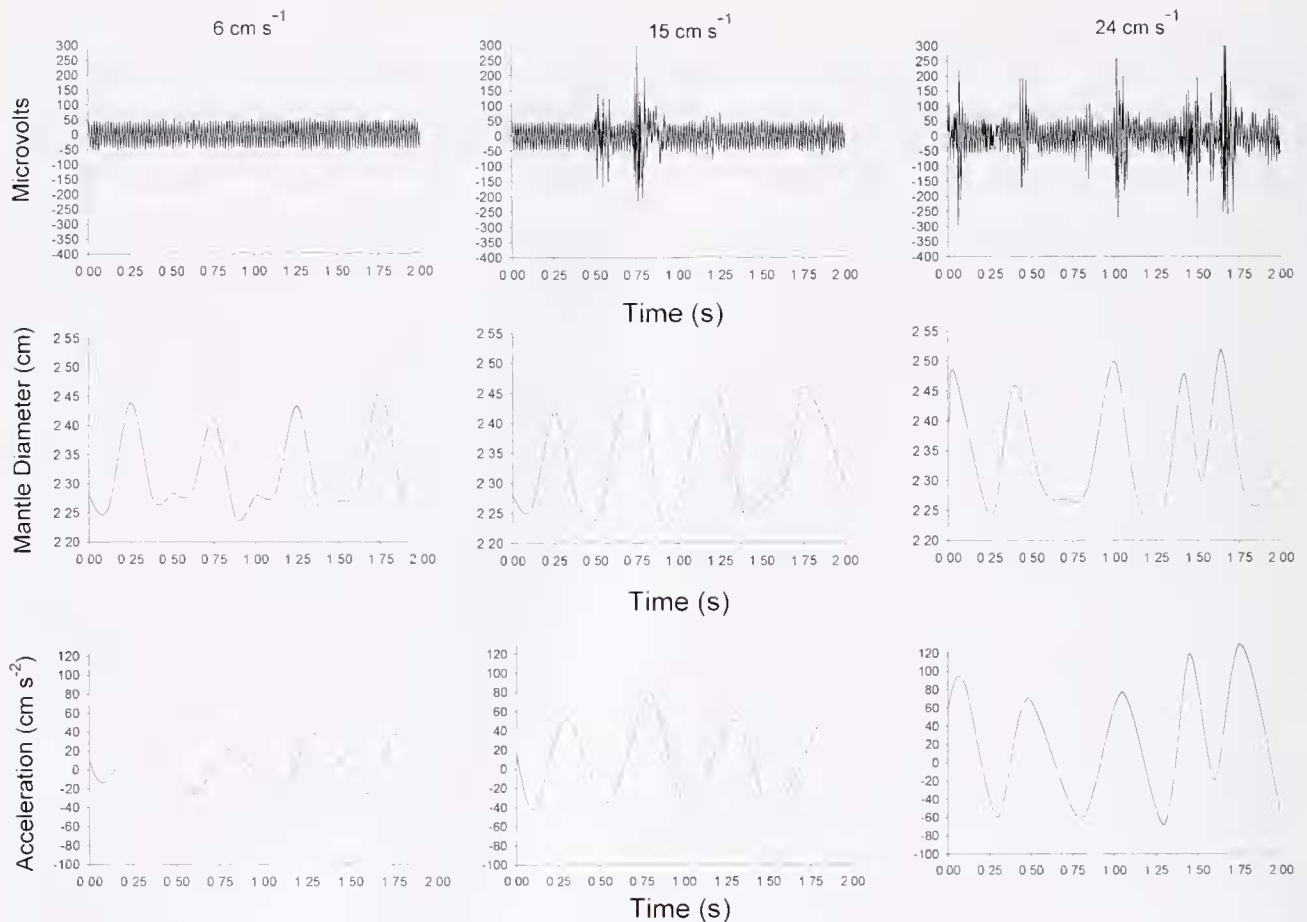


**Figure 1.** Electromyograms recorded from electrodes embedded in both peripheral aerobic circular muscle and central anaerobic circular muscle of a brief squid (5.5 cm dorsal mantle length) while swimming at 6, 15, and 24  $\text{cm s}^{-1}$ . Mantle diameter and acceleration are plotted underneath each recording. Electrical activity occurs during each mantle contraction, and at high speeds electrical activity is sometimes present during mantle expansion.

burst swimming in fishes (Rome *et al.*, 1984; Jayne and Lauder, 1996) and escape jets in cephalopods (Bone *et al.*, 1981, 1994; Mommsen *et al.*, 1981). Finke *et al.* (1996) determined that anaerobic end products, such as  $\alpha$ -glycerophosphate, succinate, and octopine, begin to accumulate in *Lolliguncula brevis* at speeds of 1.5–2.0 mantle lengths  $\text{s}^{-1}$  (8.3–11.0  $\text{cm s}^{-1}$  for a specimen of 5.5-cm DML), suggesting that anaerobic metabolism may occur in the mantle at low intermediate speeds. Moreover, intramantle pressure records revealed that at speeds of 3.2 mantle lengths  $\text{s}^{-1}$  (17.6  $\text{cm s}^{-1}$  for a specimen of 5.5-cm DML) high mantle pressures comparable to those recorded at speeds of 4.3 mantle lengths  $\text{s}^{-1}$  (23.7  $\text{cm s}^{-1}$  for a specimen of 5.5-cm DML) are periodically generated (Finke *et al.*, 1996). Accumulation of anaerobic end products and the occasional detection of high intramantle pressures at intermediate speeds are consistent with the activity of anaerobic circular muscle observed at intermediate speeds in this study. Periodic anaerobic circular mantle activity at intermediate speeds boosted power production, which helped squid keep

pace with free-stream flow in the tunnels. Periodic anaerobic activity was probably not energetically deleterious since anaerobic metabolic changes can be rapidly reversed in squid (Pörtner *et al.*, 1993). At higher speeds, central anaerobic circular muscle activity was more frequent, but there was still some oscillation between aerobic and anaerobic muscular activity. Finke *et al.* (1996) suggest that oscillating between aerobic and anaerobic circular muscle recruitment rather than simply relying exclusively on anaerobic muscle at a critical speed allows for an extended net use of anaerobic resources before fatigue sets in.

One limitation of this study was the level of background noise ( $\pm 40 \mu\text{V}$  at between 60 and 120 Hz) in EMG recordings despite attempts to reduce such interference by shielding the variable-speed flume motor and performing trials in laboratories with minimal electrical equipment. The noise probably came from interference produced by the flume motor and other electrical laboratory equipment (*e.g.*, fluorescent lights, proportional controllers, and rheostats) and was allowed through the 20–10,000-Hz bandpass filter. A



**Figure 2.** Electromyograms recorded from electrodes embedded in central anaerobic circular muscle of a brief squid (5.7 cm dorsal mantle length) while swimming at 6, 15, and 24 cm s<sup>-1</sup>. Mantle diameter and acceleration are plotted underneath each recording. There are no obvious waveforms at 6 cm s<sup>-1</sup>, occasional waveforms at 15 cm s<sup>-1</sup>, and frequent waveforms at high speeds (24 cm s<sup>-1</sup>). At high speeds, high-amplitude electrical activity occurs during each mantle contraction, and lower amplitude electrical activity sometimes occurs during mantle expansion. Furthermore, mantle contractions are more erratic at 24 cm s<sup>-1</sup> than at 6 cm s<sup>-1</sup>.

significant proportion of the noise might have been removed with a 60-Hz notch filter (Loeb and Gans, 1986), but unfortunately the electromyographic system used could not be configured with a notch filter. Considering the high level of background noise, it is possible that some low-level EMG activity was undetected. However, it is unlikely that anaerobic EMG activity was absent at low speeds because it was concealed in the noise. EMG activity from the thin peripheral (aerobic) muscle zones was easily distinguishable and consistently recorded at low speeds when muscular activity was lowest in spite of the electrical noise, and thus EMG activity from the thick, powerful central (anaerobic) muscle zone should have been visible if those fibers were active.

Although electrical noise probably did not mask circular muscle activity, which was the focus of this study, it interfered with detection of EMG activity from the smaller radial muscles. Dissections revealed that electrodes crossed radial muscle bands, which are active during mantle expansion

and hyperinflation (*i.e.*, a sharp increase in mantle diameter just prior to contraction) (Gosline *et al.*, 1983; Bone *et al.*, 1994). However, no radial EMG activity was detected at low speeds. This is not surprising given that Wilson (1960) and Ward (1972), who were the first to record electrical activity in the squid mantle musculature, were unable to detect radial muscle activity, and Gosline *et al.* (1983) and Bone *et al.* (1994) were able to detect consistent radial muscle activity in squid and cuttlefish only when using a 60-Hz notch filter and a Faraday cage, respectively. Radial muscle activity at low speeds detected by Gosline *et al.* (1983) and Bone *et al.* (1994), who examined squid and cuttlefish in dissecting pans and small aquaria and consequently did not have to contend with a motor-driven swim tunnel, was often less than 40  $\mu$ V. Therefore, low-speed radial muscle activity presumably was hidden by the electrical noise. From squid engaged in escape jets, Gosline *et al.* (1983), without using a 60-Hz notch filter, recorded

radial muscle activity during refilling and hyperinflation. At high speeds, EMG activity frequently was observed in the present study during mantle expansion. Since circular muscle is not active during mantle expansion (Gosline *et al.*, 1983), radial muscles—the only other muscle into which electrodes were imbedded—were probably responsible for the EMG activity observed during mantle refilling. The detection of radial muscle activity at high speeds when anaerobic circular muscle was active suggests that significant radial muscle activity is used to expand the mantle during vigorous jetting. Elastic energy stored within the connective tissue of the mantle also probably plays an important role during mantle expansion (Gosline and Shadwick, 1983; Gosline *et al.*, 1983; Pabst, 1996).

Using electromyography, Kier *et al.* (1989) demonstrated that anaerobic and aerobic zones of muscle in cuttlefish *Sepia officinalis* fins have distinct functional roles, with aerobic fibers responsible for gentle fin movements and anaerobic muscles responsible for vigorous fin movements and support for that movement. The results of the present study indicate that peripheral aerobic circular muscle and central anaerobic circular muscle in brief squid *Lolliguncula brevis* also have distinct functional roles that are analogous to red and white myotomal fibers in fishes. Contrary to the situation in many fishes, however, in squids the anaerobic muscle appears to be used at subcritical speeds. The discovery of functional “gears” in squids, which is yet another instance of convergent evolution in fishes and cephalopods, is significant because it suggests that there is specialization and efficient use of locomotive muscle in squids. Future study on the innervation and neural control of these different muscle fibers during unrestrained swimming should provide further insight into the locomotive system of cephalopods.

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### Literature Cited

- Bartol, I. K. 1999.** *Distribution, Swimming Physiology, and Swimming Mechanics of the Brief Squid Lolliguncula brevis*. Ph.D. dissertation, College of William and Mary, Virginia Institute of Marine Science, Gloucester Point, VA. 215 pp.
- Bone, Q. 1966.** On the function of the two types of myotomal muscle fibers in elasmobranch fish. *J. Mar. Biol. Assoc. UK* **46**: 321–349.
- Bone, Q. 1978.** Locomotor muscle. Pp. 361–424 in *Fish Physiology*, vol. 7, W. S. Hoar and D. J. Randall, eds. Academic Press, New York.
- Bone, Q., A. Pulsford, and A. D. Chub. 1981.** Squid mantle muscle. *J. Mar. Biol.* **61**: 327–342.
- Bone, Q., E. R. Brown, and G. Travers. 1994.** On the respiratory flow in the cuttlefish *Sepia officinalis*. *J. Exp. Biol.* **194**: 153–165.
- Coughlin, D. J., L. Valdes, and L. C. Rome. 1996.** Contraction dynamics and power production of pink muscle of scup (*Stenotomus chrysops*). *J. Exp. Biol.* **199**: 2703–2712.
- Curtin, N. A., and R. C. Woledge. 1988.** Power output and force-velocity relationships of live fibers from white myotomal muscle of the dogfish. *Scyliorhinus canicula*. *J. Exp. Biol.* **140**: 187–197.
- Fields, J. H. A., and J. F. Quinn. 1981.** Some theoretical considerations on cytosolic redox balance during anaerobiosis in marine invertebrates. *J. Theor. Biol.* **88**: 35–45.
- Finke, E., H. O. Pörtner, P. G. Lee, and D. M. Webber. 1996.** Squid (*Lolliguncula brevis*) life in shallow waters: oxygen limitation of metabolism and swimming performance. *J. Exp. Biol.* **199**: 911–921.
- Flitney, F. W., and I. A. Johnston. 1979.** Mechanical properties of isolated fish red and white muscle fibers. *J. Physiol.* **295**: 49–50.
- Gosline, J. M., and R. E. Shadwick. 1983.** The role of elastic energy storage mechanisms in swimming: an analysis of mantle elasticity in escape jetting in the squid, *Loligo opalescens*. *Can. J. Zool.* **61**: 1421–1431.
- Gosline, J. M., J. D. Steeves, A. D. Harman, and E. DeMont. 1983.** Patterns of circular and radial mantle muscle activity in respiration and jetting of the squid *Loligo opalescens*. *J. Exp. Biol.* **104**: 97–109.
- Grieshaber, M. E., and G. Gade. 1976.** The biological role of octopine in the squid, *Loligo vulgaris* (Lamarck). *J. Comp. Physiol.* **108**: 225–232.
- Hochachka, P. W., T. W. Moon, T. Mustafa, and K. B. Storey. 1975.** Metabolic sources of power for mantle muscle of a fast swimming squid. *Comp. Biochem. Physiol.* **52B**: 151–158.
- Jayne, B. C., and G. V. Lauder. 1996.** New data on axial locomotion in fishes: how speed affects diversity of kinematics and motor patterns. *Am. Zool.* **36**: 642–655.
- Johnston, I. A., W. Davison, and G. Goldspink. 1977.** Energy metabolism of carp swimming muscles. *J. Comp. Physiol.* **114**: 203–216.
- Kier, W. M. 1988.** The arrangement and function of molluscan muscle. Pp. 211–252 in *The Mollusca, Form and Function*, vol. 11, E. R. Trueman and M. R. Clarke, eds. Academic Press, New York.
- Kier, W. M., and K. K. Smith. 1985.** Tongues, tentacles and trunks: the biomechanics of movement in muscular hydrostats. *Zool. J. Linn. Soc.* **83**: 307–324.
- Kier, W. M., K. K. Smith, and J. A. Mian. 1989.** Electromyography of the fin musculature of the cuttlefish *Sepia officinalis*. *J. Exp. Biol.* **143**: 17–31.
- Loeb, G. E., and C. Gans. 1986.** *Electromyography for Experimentalists*. University of Chicago Press, Chicago.
- Messinger, J. B., M. Nixon, and K. P. Ryan. 1985.** Magnesium chloride as an anaesthetic for cephalopods. *Comp. Biochem. Physiol.* **82C**: 203–205.
- Mommsen, T. P., J. Ballantyne, D. MacDonald, J. Gosline, and P. W. Hochachka. 1981.** Analogues of red and white muscle in squid mantle. *Proc. Natl. Acad. Sci. USA* **78**: 3274–3278.
- O'Dor, R. K. 1982.** The respiratory metabolism and swimming performance of the squid, *Loligo opalescens*. *Can. J. Fish. Aquat. Sci.* **39**: 580–587.
- O'Dor, R. K. 1988a.** The forces acting on swimming squid. *J. Exp. Biol.* **137**: 421–442.

- O'Dor, R. K. 1988b. Limitations on locomotor performance in squid. *J. Appl. Physiol.* **64**: 128–134.
- Otis, T. S., and W. F. Gilly. 1990. Jet-propelled escape in the squid *Loligo opalescens*: concerted control by giant and non-giant motor axon pathways. *Proc. Natl. Acad. Sci. USA* **87**: 2911–2915.
- Pabst, D. A. 1996. Springs in swimming animals. *Am. Zool.* **36**: 723–735.
- Pörtner, H. O., D. M. Webber, R. G. Boutilier, and R. K. O'Dor. 1991. Acid-base regulation in exercising squid (*Illex illecebrosus*, *Loligo pealeii*). *Am. J. Physiol.* **261**: R239–R246.
- Pörtner, H. O., D. M. Webber, R. K. O'Dor, and R. G. Boutilier. 1993. Metabolism and energetics in squid (*Illex illecebrosus*, *Loligo pealeii*) during muscular fatigue and recovery. *Am. J. Physiol.* **265**: R157–R165.
- Preuss, T., and W. F. Gilly. 2000. Role of prey-capture experience in the development of the escape response in the squid *Loligo opalescens*: a physiological correlate in an identified neuron. *J. Exp. Biol.* **203**: 559–565.
- Rome, L. C., P. T. Loughna, and G. Goldspink. 1984. Muscle fiber activity of carp as a function of swimming speed and muscle temperature. *Am. J. Physiol.* **247**: R272–R279.
- Smith, K. K., and W. M. Kier. 1989. Trunks, tongues and tentacles: moving with skeletons of muscle. *Am. Sci.* **77**: 28–35.
- Storey, K. B., and J. M. Storey. 1978. Energy metabolism in the mantle muscle of the squid, *Loligo pealeii*. *J. Comp. Physiol.* **123**: 169–175.
- Vogel, S., and M. LaBarbera. 1978. Simple flow tanks for research and teaching. *BioScience* **10**: 638–643.
- Ward, D. V. 1972. Locomotory function of the squid mantle. *J. Zool. (Lond)*. **167**: 487–499.
- Wilson, D. M. 1960. Nervous control of movement in cephalopods. *J. Exp. Biol.* **37**: 57–72.